Hydrolysis of Prosulfuron at pH 5: Evidence for a Resonance-Stabilized Triazine Cleavage Product

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(Received 22 April 1996; revised version received 18 February 1997; accepted 8 April 1997)

Abstract: Prosulfuron is a herbicide for the selective control of broadleaf weeds in corn. In order to examine the effect of pH on the stability of prosulfuron in aqueous solution, a hydrolysis experiment was conducted in buffer at pH 5, 7 and 9 utilizing [*phenyl*-^{14}C] and [*triazine*-^{14}C]prosulfuron. Prosulfuron was found to be stable under neutral and basic conditions and hydrolytically unstable under acidic conditions ($T_{\frac{1}{2}} \sim 10$ days). One of the major routes of degradation at pH 5 involved hydrolysis of the sulfonylurea bridge to yield the corresponding phenyl- and triazine-cleavage products and minor amounts of desmethyl-prosulfuron and dihydroxymethyltriazine. In addition to these hydrolysis products, an unknown species was observed at significant levels after 30 days ($\sim$20% of the applied dose). A large-scale experiment was subsequently performed to generate additional material for the spectroscopic characterization and identification of this unknown degradate. A thorough spectral evaluation of this unknown revealed a resonance-stabilized derivative of the parent molecule which stemmed from a hydrolytic opening of the triazine ring of desmethyl-prosulfuron. Although this triazine ring-opening phenomenon was first described for chlorsulfuron, a structurally related sulfonylurea herbicide, the structure of the product was only postulated. Recently, Cambon *et al.* described the hydrolysis kinetics of thifensulfuron and thifensulfuron-methyl which resulted in cleavage of the sulfonylurea bridge and triazine ring-opening. Our results support these findings and we herein describe the characterization and identification of this resonance-stabilized species by comparison with the recently synthesized authentic reference standard.

Pestic Sci., **51**, 56–64, 1997
No. of Figures: 6. No. of Tables: 0. No. of Refs: 6

Key words: prosulfuron, sulfonylurea, hydrolysis, triazine

1 INTRODUCTION

Prosulfuron (Fig. 1, **1**) belongs to the sulfonylurea class of herbicides which were initially discovered in the mid-1970s.[1] In the case of prosulfuron, the heterocycle portion of the molecule is a substituted 1,3,5-triazine, which independently exhibits biological activity in the absence of the sulfonylurea linkage. Our main goal was to determine the environmental fate of **1**. During the course of a hydrolysis experiment at pH 5, the expected phenyl and triazine bridge cleavage products were observed arising from hydrolysis of the sulfonylurea

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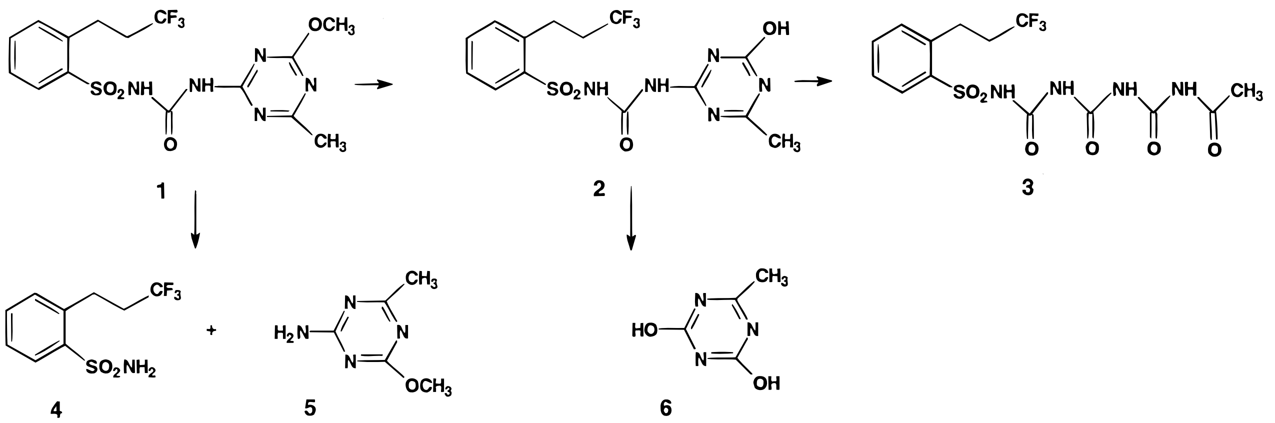

Fig. 1. Hydrolysis of prosulfuron at pH 5.

linkage (Fig. 1). In addition to the cleavage products, minor amounts of desmethyl-prosulfuron and dihydroxymethyltriazine were observed. Identification of these degradates was possible by comparison of the degradate spectra with the authentic reference standard spectra (MS and NMR). Unexpectedly, an unknown degradate formed with similar polarity to the parent molecule. The structure of this unknown was proposed as **3** after isolation, purification and subsequent spectral evaluation by mass spectrometry and [^{1}H]NMR. Structural confirmation became possible after synthesis of the authentic reference standard for spectral comparison.[2]

During the early stages of our environmental assessment of prosulfuron, Reiser *et al.*[3] postulated the existence of a degradate which resulted from ring-opening of the 1,3,5-triazine substituent of chlorsulfuron. Chlorsulfuron, a sulfonylurea herbicide, bears the same 2-amino-4-methoxy-6-methyltriazine substituent as **1**. The degradation pathway proposed by Reiser is the same as outlined for **1** (Fig. 1), but, in the absence of a synthetic standard for the hypothesized ring-opened structure analogous to **3**, structural confirmation of the degradate was not possible. Other groups of workers have also postulated degradates resulting from triazine ring-opening, but these structures were never confirmed by isolation and confirmation of the isolate structure with the authentic reference standard.[4,5] The kinetics of hydrolysis have only recently been described for thifensulfuron and thifensulfuron-methyl.[6]

This report outlines the identification of **3** as well as structural confirmation of the hydrolysis degradates resulting from hydrolysis of the sulfonylurea bridge.

2 MATERIALS AND METHODS

2.1 Test materials and reference standards

Prosulfuron (**1**), 1-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)-3-[2-(3,3,3-trifluoropropyl)phenylsulfonyl]urea, labeled uniformly in the phenyl ring and uniformly in the triazine ring was prepared by the Chemical Synthesis Group, Biochemistry Resource Department, Ciba-Geigy Corp., with specific activities of 32·5 μCi mg^{-1} (phenyl label) and 35·3 μCi mg^{-1} (triazine label). The radiochemical purities for the phenyl label and triazine label were 100% and 99·6%, respectively. Reference standard **3**, *N*-[[[2-(3,3,3-trifluoropropyl)phenylsulfonylcarbamoyl]carbamoyl]carbamoyl]acetamide,[2] and all other unlabeled authentic reference standards (**2**, **4**, **5** and **6**) were also synthesized by the Ciba Chemical Synthesis Group. All hydrolysis studies were conducted with HPLC grade water.

2.2 Hydrolysis experiment

Three hydrolysis experiments (pH 5, 7 and 9) were performed with phenyl- and triazine-labeled prosulfuron at a concentration of approximately 10 μg ml^{-1}. Each experiment was conducted by adding an aliquot (287 μl) of either triazine- or phenyl-radiolabeled prosulfuron stock solution in acetonitrile (11 mg ml^{-1}) to sterile buffered solution (325 ml) at pH 5 (0·01 M HOAc/NaOAc), pH 7 (0·01 M KH_2PO_4/Na_2HPO_4) and pH 9 (0·01 M $Na_2B_4O_7$/HCl). After sonication, triplicate 100-ml aliquots of each test solution were aseptically transferred to silylated, sterile, foil-covered 100-ml Erlenmeyer flasks. The flasks were stoppered with sterile polyurethane foam plugs and incubated in a Fisher Scientific Low Temperature Incubator at a temperature of 25($\pm$1)°C. Three-millilitre subsamples were aseptically removed from each triplicate flask on days 0, 1, 3, 7, 15, 20, 24 and 30 and subjected to radioassay by liquid scintillation counting (LSC) and high pressure liquid chromatography (HPLC) analysis.

2.2.1 HPLC and TLC analysis of hydrolysis products

Pooled sample solutions were analyzed by injecting 50-μl aliquots of each solution directly onto an HPLC system with a Hewlett-Packard HP1050 quaternary pump equipped with either a Supelcosil LC-18 reverse phase column (25 cm × 4·6 mm ID, 5 μm particle size, Supelco Inc.) or a Chromanetics Partisil ODS-3 reverse

phase column of the same size (P.J. Assoc.). The eluting compounds were detected with a Radiomatic Beta Flo-One radioactive flow detector (Radiomatic Instruments) and a Hewlett-Packard Model 1050 variable wavelength UV/Vis detector or diode array variable wavelength UV/Vis detector (280 nm). The mobile phase for the linear gradient (1 ml min^{-1}) consisted of acetonitrile (Solvent A) and HPLC grade water adjusted to pH 2·5 with 85% H_3PO_4 (Solvent B). From 0 to 15 min Solvent A was ramped from 0 to 30%. From 15 to 40 min, Solvent A was increased from 30% to 60%. From 40 to 45 min, Solvent A was ramped to 100% which was held for 5 min.

The ^{14}C-components in the hydrolysates were monitored by UV and carbon-14 detection. The parent had a retention time of ~37 min. The degradate retention times were as follows: **3** (~30 min), **4** (~28 min), **2** (~26 min), **5** (~12 min) and **6** (~5 min).

Thin layer chromatography was used to augment the HPLC characterization of the hydrolysates. Representative samples were chromatographed on Merck 254-F silica gel plates (0·25 mm thickness, EM Science). Co-chromatography was performed by co-spotting a solution of reference standards with the hydrolysate. The plates were developed in two dimensions: (1) ethyl acetate + ethanol + 14·8 M ammonium hydroxide + water (40 + 20 + 2 + 2 by volume) and (2) butanol + 17·4 M glacial acetic acid + water (4 + 1 + 1 by volume). The reference standards were visualized by UV light. The radioactivity was visualized by a Bioscan Series 200A imaging scanner (Bioscan, Inc.). For quantitation, the plates were marked into zones and the zones were scraped. Individual zones were dissolved in methanol (1 ml) and after approximately 1 h, scintillation cocktail was added (10 ml) and the fractions were radioassayed.

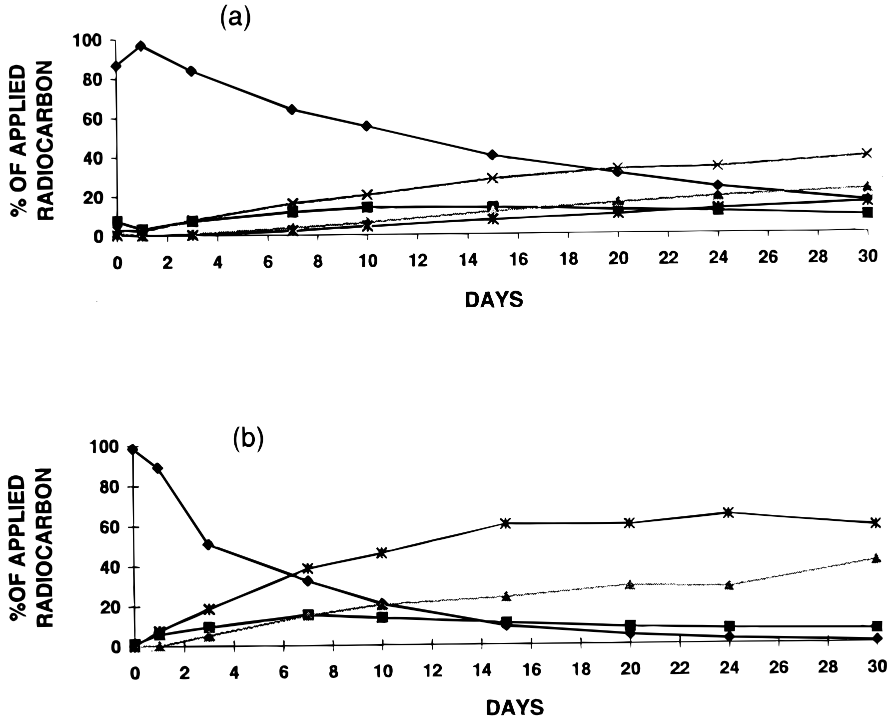

Fig. 2. Hydrolysis of (a) [*triazine*-^{14}C]prosulfuron and (b) [*phenyl*-^{14}C]prosulfuron. (◆) **1**; (■) **2**; (▲)**3**; (×) **5**; (*) (a) **6**, (b) **4**.

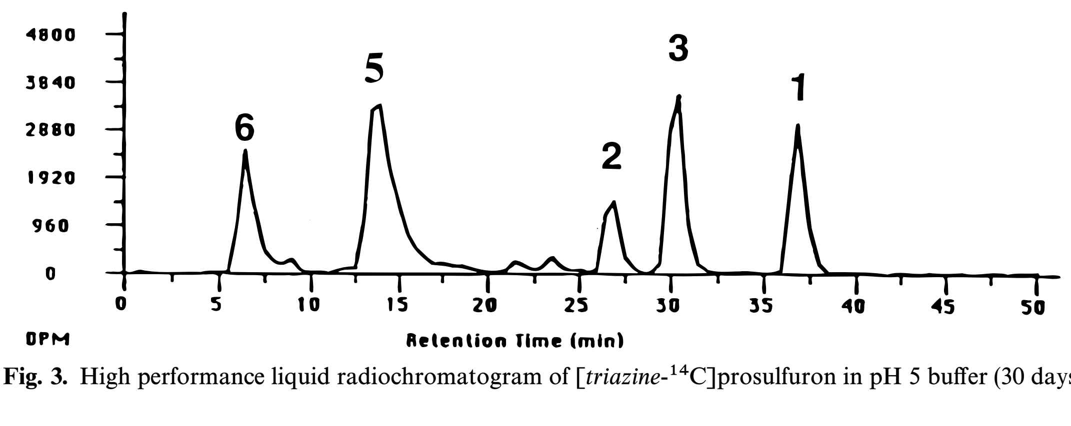

Fig. 3. High performance liquid radiochromatogram of [*triazine*-^{14}C]prosulfuron in pH 5 buffer (30 days).

2.3 Preparation and identification of 1, 2, 4 and 5

A scale-up experiment was performed for both the triazine and phenyl labels at pH 5 in order to generate additional material for identification of degradates. For the triazine label, 10·3 ml of [^{14}C]prosulfuron in acetonitrile (1·48 mg ml^{-1}) was aseptically added to 1489·7 ml of pH 5 sterile buffer in a 2-litre foil-covered Erlenmeyer flask equipped with a sterile polyurethane foam plug. For the phenyl label, 1·7 ml of [^{14}C]prosulfuron in acetonitrile (8·84 mg ml^{-1}) was aseptically added to 1498·3 ml of sterile pH 5 buffer in a foil-covered Erlenmeyer flask equipped with a sterile polyurethane foam plug. Each label experiment was incubated at 26·2($\pm$2)°C and aliquots from each label were removed for radioassay and HPLC analysis at the following intervals: 0, 1, 5, 8, 12, 19, 23 and 30 days. The day 0 and day 12 phenyl-label samples were analyzed by two-dimensional TLC (described above) and the regions corresponding to **1** (day 0) and **4** (day 12) were isolated for mass spectral analysis. For the triazine label, the day 0 and day 12 samples were applied to TLC and the regions corresponding to **1** (day 0) and **5** (day 12) were isolated for mass spectrometry.

2.3.1 Identification of 1

The presence of **1** was confirmed in the study samples by analysis with a Hewlett-Packard Model 5988A quadrupole mass spectrometer equipped with a direct inlet probe. The analyses were made in the positive ion, chemical ionization mode using methane as the ionizing gas. The temperature program used for the direct inlet probe was as follows: initial temperature, 40°C (1 min); final temperature, 300°C (6·2 min); rate, 50°C min^{-1}. The MS parameters were as follows: ion source temp., 100°C; electron multiplier, 2300 V; electron energy, 230 eV or 190 eV; emission current, 300 mA; scanning mode, 60–490 amu. The authentic prosulfuron standard and the isolated prosulfuron gave virtually identical spectra showing ions at *m/z* 420 (M + 1)$^+$, 280, 237 and 141.

2.3.2 Identification of 2

The identity of **2** (1-(4-hydroxy-6-methyl-1,3,5-triazin-2-yl)-3-[2-(3,3,3-trifluoropropyl)phenylsulfonyl]urea) was based on co-chromatography with the authentic reference standard. Co-chromatography was performed by HPLC and also with two-dimensional TLC (see above for TLC and HPLC analysis of hydrolysates). Efforts to isolate this degradate by TLC were unsuccessful due to the strong adsorption of the compound to silica gel. Since **2** has been identified in other contemporary environmental fate studies with prosulfuron, purification by alternative methods was not pursued.

2.3.3 Identification of 4

The presence of **4** (2-(3,3,3-trifluoropropyl)benzensulfonamide) in the pH 5 hydrolysate was confirmed after GC-MS analysis of the isolated radiocarbon and comparison with the authentic reference standard. The GC-MS analysis was conducted with a Hewlett-Packard Model 5988A quadrupole mass spectrometer equipped with a Hewlett-Packard Model 5890 Series II Gas Chromatograph. The MS parameters were as follows: scan range, 40–300 amu; ion source temperature, 150°C; electron impact voltage, 70 eV; emission current, 300 mA; electron multiplier voltage, ~2300 V. The GC was equipped with a Hewlett-Packard DB-1 column (30 m × 0·25 mm ID). The temperature program was as follows: initial temperature, 85°C (2 min); final temperature, 260°C (2 min); temperature ramp, 20°C min^{-1}. The helium carrier gas flow rate was 1–2 ml min^{-1} at approximately 4 psi and the injector and transfer line temperatures were 240°C and 260°C, respectively. A strong molecular ion of *m/z* 253 was observed for both the isolate and the reference standard of **4**. In addition, the spectra of the standard and the isolate were virtually identical, both showing additional ions at *m/z* 172, 152 and 109. GC retention times were 14·62 min and 14·65 min for the reference standard of **4** and the isolated **4**, respectively.

2.3.4 Identification of 5

The presence of **5** (4-methoxy-6-methyl-1,3,5-triazin-2-amine) was confirmed by spectral comparison of the isolated degradate and the authentic reference standard. Analyses were made by GC-MS in the EI mode with a Hewlett-Packard Model 5988A Quadrupole Mass Spectrometer equipped with a Hewlett-Packard Model 5890 Series II Gas Chromatograph. The MS parameters were as follows: scan range, 50–400 amu; ion source temperature, 150°C; electron impact voltage, 70 eV; emission current, 300 mA; electron multiplier voltage, 2255–2355 V. The gas chromatographic conditions were as follows: HP-1 (Hewlett-Packard) column, 10 m × 0·20 mm ID; He carrier gas, 1–2 ml min^{-1} (4 psi); injector temperature 240°C; transfer line temperature, 260°C; oven temperature program, 85°C (2 min), then 20°C min until 260°C (2 min). Both the reference standard spectrum of **5** and the isolated **5** showed a strong molecular ion at *m/z* 140 (M$^+$). Additional ions were observed in both spectra: *m/z* 110, 83, 69 and 42.

2.4 Preparation and identification of 6

A solution of [*triazine*-^{14}C]prosulfuron was prepared in pH 5 sterile buffer (5 litre) to give a final concentration of 21 μg ml^{-1}. This solution was incubated for 52 days at approximately 25°C. This solution and authentic **6** were analyzed by electrospray LC-MS utilizing a PE Sciex API 1 single quadruple mass spectrometer equipped with an Ionspray Liquid Introduction interface, and HPLC (Perkin-Elmer Model 410). The M + 1 ion at *m/z* 128 was monitored for both the reference

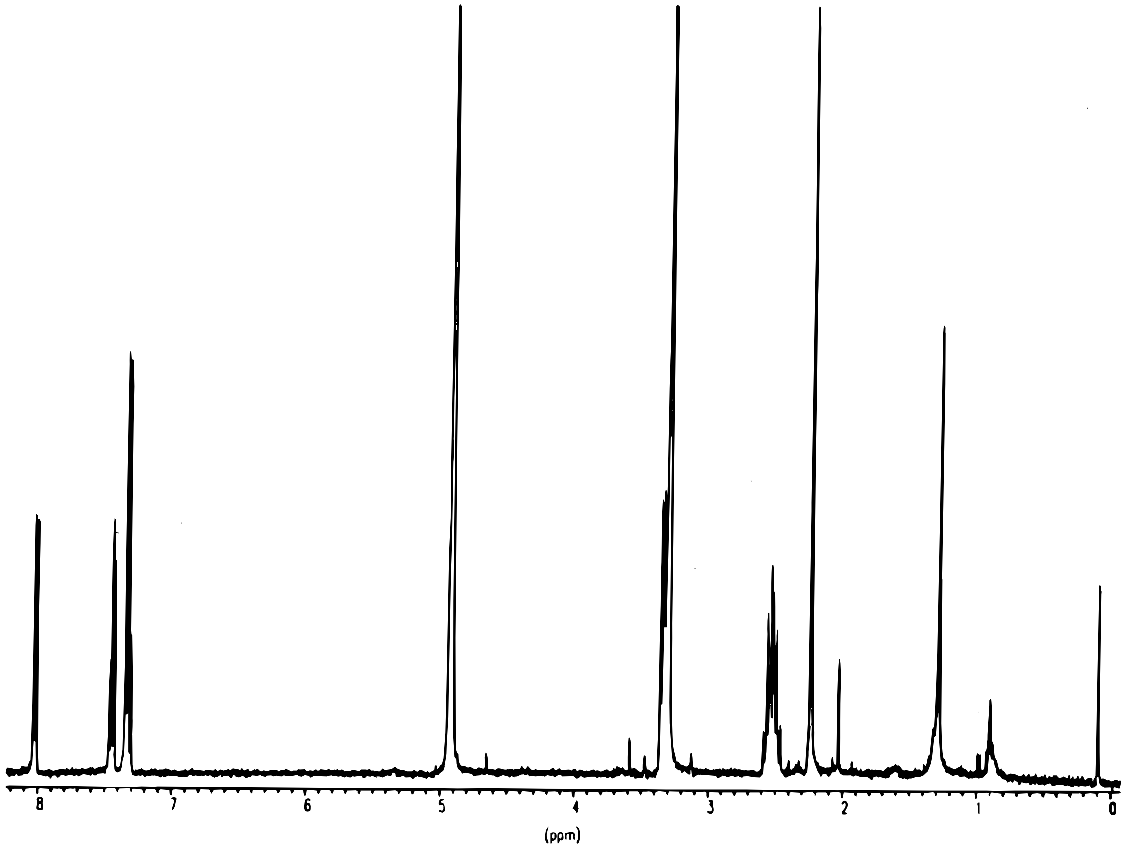

Fig. 4. [^{1}H]NMR spectrum of isolated **3**.

standard and the pH 5 hydrolysate. Both the standard and **6** generated in the incubation gave a retention time match of 2·6 min. Other parameters were as follows: interface heater, 70°C; mobile phase split ratio, 20–25 : 1; curtain gas flow, 1·1 on gauge; nebulizer gas flow, 1·2 on gauge. The HPLC conditions for the identification of **6** were as follows: column heater, 30°C; injection volume, 100 μl; flow rate, 1 ml min^{-1}; column, Zorbax R × C8, 15 cm × 4·6 mm ID; Solvent A, acetic acid + acetonitrile (0·1 + 99·9 by volume); Solvent B, acetic acid + water (0·1 + 99·9 by volume). The linear gradient program was initiated with 100% Solvent B which was held for 2 min. From 2 to 10 min, Solvent B was switched to 100% A which was held for 3 min (10–13 min with 100% Solvent A). The reference standard of **6** [2,4-dihydroxy-6-methyl-1,3,5-triazine] and **6** contained in the hydrolysate both gave strong molecular ions (M + 1 = 128) in the scanning mode.

2.5 Preparation, purification and NMR analysis of isolated 3 and reference standard 1

In order to generate milligram quantities of **3** for NMR and MS analysis, unlabeled prosulfuron (50·2 mg) was fortified with a tracer quantity of [*triazine*-^{14}C]prosulfuron (6 058 000 dpm, 35.3 μCi mg^{-1}) in 5 ml acetonitrile. This solution was diluted to 2·3 litre with sterile pH 5 buffer and incubated in the dark at 25($\pm$0)°C. After 32 days, the solution was extracted with ethyl acetate, concentrated *via* rotary evaporation, and the concentrated extract was passed through a plug of sodium sulfate to remove residual water. A final concentration of the extract was made down to 1·5 ml.

2.5.1 *TLC purification of 3*

The concentrated extract (triazine label) was applied as a band on two silica gel TLC plates (2 mm thickness, Merck Silica Gel 60, 254-F). The TLC plates were eluted once using dichloromethane + methanol + deuterated acetic acid (90 + 10 + 10 by volume). The band corresponding to **3** had R_f values of 0·68, 0·70 and 0·39 were obtained for **1**, **5** and **2**, respectively, in this system. The silica gel region containing **3** was scraped from the plate and eluted with ethyl acetate + methanol (1 + 1, by volume) by filtering over a pad of Celite®. This solution was concentrated to dryness and reconstituted in acetonitrile. The solution was filtered again using a Gelman Acrodisc CR PTFE filter (0·22 μm, Gelman Sciences), concentrated *via* rotary evaporation and diluted in deutero-acetonitrile. Crystallization of **3** occurred after freezer storage of this solution.

2.5.2 *NMR analysis of 3*

Isolation of **3** as a crystalline solid was performed by decanting the deuteroacetonitrile solution, evaporating

the residual acetonitrile with a stream of nitrogen, and dissolving the remaining solid in deuteromethanol. [^{1}H]NMR was run with a Bruker AMX-400 NMR spectrometer (17°C) with the following acquisition parameters: 32 768 data points, 4807·7 Hz sweep width and 8·0 μs pulse width. The instrument was locked onto residual methanol (δ 3·30) relative to tetramethylsilane (δ 0·00). The chemical shifts are reported in ppm values (δ) and J values are given in Hertz. The following proton multiplicities are abbreviated as follows: s = singlet, d = doublet, t = triplet and m = multiplet. The synthetic reference standards of parent prosulfuron and **3** were also run in deuteromethanol using the acquisition parameters described above.

[^{1}H]NMR of isolated **3**: δ 2·24 (s, 3H, CH_3), 2·52 (m, 2H, $C\underline{H}_2CF_3$), 3·33 (m, 2H, $ArCH_2$), 7·33 (overlapping ddd, dd, J = 8·4, 1·4 Hz, 2 ArH), 7·44 (ddd, J = 7·4, 1·4 Hz, 1 ArH), 8·23 (dd, J = 7·9, 1·1 Hz, 1 ArH).

[^{1}H]NMR of reference standard **3**: δ 2·14 (s, 3H, CH_3), 2·53 (m, 2H $C\underline{H}_2CF_3$), 3·29 (m, 2H, $ArCH_2$, obscured by methanol protons), 7·48 (m, 2 ArH), 7·64 (ddd, J = 7·4, 1·9 Hz, 1 ArH), 8·11 (dd, J = 8·0, 1·3 Hz, 1 ArH).

[^{1}H]NMR of prosulfuron standard: δ 2·51 (s, 3H, CH_3), 2·55 (m, 2H, CH_2CF_3), 3·30 (m, 2H, $ArCH_2$), 4·02 (s, 3H, OCH_3), 7·48 (overlapping ddd, dd, J = 9·0, 1·6 Hz, 2 ArH), 7·65 (ddd, J = 7·9, 1·4 Hz, 1 ArH), 8·15 (dd, J = 7·8, 1·0 Hz, 1 ArH).

2.6 FAB-MS analysis of the isolated 3

Both negative and positive ion FAB-MS analysis of **3** was performed with a Hewlett-Packard Model 5988A quadrupole mass spectrometer equipped with a Chemstation® Data System. The following instrument parameters were utilized: ion source temperature, ambient; inlet, FAB probe; scan range, 50–500 amu; discharge current, 30 μA; Xe beam energy, 5–7 kV; electron multiplier voltage, 2528 V. Spectra were obtained in a glycerol + methanol (1 + 1 by volume) matrix. Data were acquired over a 5-min period and the spectra were averaged over the total ion trace (50–500 *m/z*).

The negative and positive FAB mass spectra of isolated **3** clearly showed molecular ions at *m/z* 423 $(M - H)^-$ and 425 $(M + H)^+$, respectively. Additional ions were observed at *m/z* 445 (M + Na), *m/z* 295 [M − $(CONH)_2COCH_3$)] and *m/z* 252 [M − $(CONH)_3COCH_3$] in the negative ion mode.

2.7 Electrospray LC-MS analysis of the isolated 3 and the authentic analytical reference standard of 3

Analyses utilized a PE Sciex API 1 single quadrupole mass spectrometer equipped with an Ionspray Liquid Introduction interface, and HPLC (Perkin-Elmer Model 410). The isolate was scanned in the negative ion mode from *m/z* 250–450 or 200–600 with an orifice voltage of either −50 V (molecular ion information only) or −100 V (additional fragmentation). The reference standard (synthesized and analyzed at a later date) was analyzed using the same parameters as the isolate except the orifice voltage was set at −60 V. Other parameters are as follows: interface heater, 70°C;

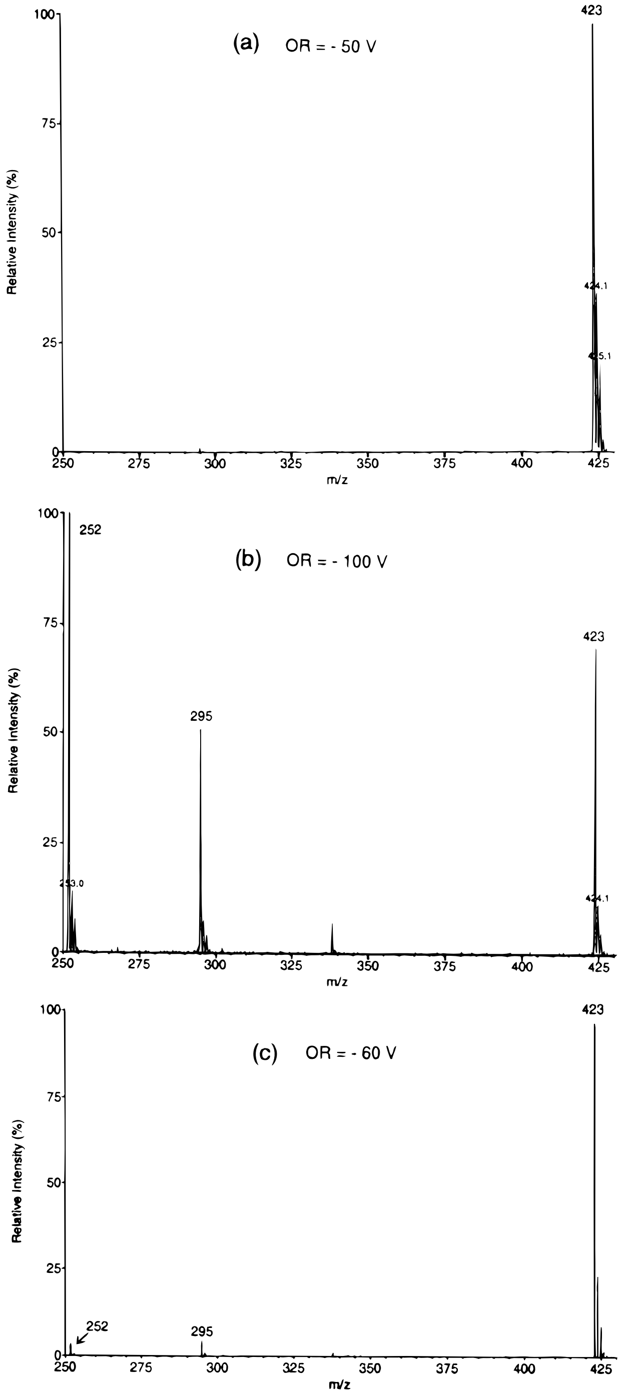

Fig. 5. Electrospray LC/MS spectra of: (a) isolated 3 at an orifice potential of −50 V, (b) isolated 3 at −100 V to induce fragmentation and (c) synthetic reference standard (−60 V).

mobile phase split ratio, 20–25 : 1; curtain gas flow, 1·1 on gauge; nebulizer gas flow, 1·2 on gauge. The HPLC conditions for the remaining analytes are as described as follows: column heater, 30°C; injection volume, 100 μl; flow rate, 1 ml min^{-1}; column, Zorbax R × C8 (15 cm × 4·6 mm ID); Solvent A, acetic acid + acetonitrile (0·1 + 99·9 by volume), Solvent B, acetic acid + acetonitrile + water (0·1 + 30 + 69·9 by volume). The linear gradient program went from 100% Solvent B to 100% Solvent A over 0–10 min. Solvent A was held at 100% from 10 to 15 min followed by equilibration back to 100% B from 15 to 16 min and a hold on Solvent B for 5 min.

Using an orifice voltage of −50 V, the electrospray MS spectrum of isolated **3** showed a molecular weight of *m/z* 423 $(M-H)^-$ with no additional fragmentation. Additional fragmentation was observed, however, when the orifice potential was increased to −100 V. Ions at *m/z* 295 $[M-(CONH)_2COCH_3]$, and *m/z* 252 $[M-(CONH)_3COCH_3]$ were present in addition to the molecular ion at *m/z* 423 $(M-H)^-$. The LC retention time was 6·68 min.

Analysis of reference standard **3** was initially performed by infusion of the concentrated standard at an orifice voltage of −60 V (negative ion mode). Due to the high concentration, the molecular ion [*m/z* 423 (M − 1)] as well as minor fragmentation (*m/z* 295 and 252) was observed. In a later experiment, the sample was introduced *via* the HPLC column using the same conditions and mobile phase as described above. Under these conditions, an orifice potential of −60 V gave only the molecular ion [*m/z* 423 (M − 1)]. An HPLC retention time of 6·42 min was obtained.

2.8 Positive Ion FAB-high resolution analysis of 3

Analyses were conducted at Mass Search, Inc. (Modesto, CA) using a VGZAB2SE high resolution double focusing mass spectrometer. The exact mass measurement was made using the peak matching module on the instrument at a resolution of 10 000 : 1. The liquid matrix for this measurement was an aqueous 10% v/v solution of polyethylene glycol (PEG) covering the mass range from 200 to 700 Daltons. The PEG ion used for the measurement was 425·073 423 $(M+H)^+$. These data establish a mass of 424·073 423 for **3** (assuming an intact CF_3 moiety and an even number of nitrogens) and an empirical formula of $C_{14}H_{16}O_6N_4SF_3$.

2.9 FTIR of the isolated 3 and prosulfuron standard

Infrared spectra were recorded on a Mattson Cygnus 100 FTIR Spectrophotometer. The prosulfuron standard and isolated **3** were each dissolved in acetonitrile and applied to a salt plate as a thin film. Comparison of the IR of the isolated **3** and standard **1** revealed the absence of the triazine bands (~1560 cm^{-1}) in **3** as compared to parent prosulfuron.

IR of **3** (salt plate): 3170, 2910, 1750, 1720, 1660, 1455, 1250, 1130 cm^{-1}. IR of prosulfuron standard (salt plate): 3010, 1720, 1560, 1455, 1350, 1140 cm^{-1}.

3 RESULTS AND DISCUSSION

The hydrolytic decline in prosulfuron concentrations at pH 5 (25°C) led to a concomitant rise in the amount of **5** and the corresponding bridge cleavage product (**4**) (Fig. 2). A less prominent, yet significant, degradation pathway involved *O*-demethylation of the parent molecule to yield **2**. Maximum levels of **2** were observed on day 10 in the triazine study (13·9% of the applied radiocarbon) and day 7 in the phenyl study (15·4% of applied). The decline of **2** was followed by a concomitant increase in the formation of **3** which suggests that **2** is the precursor to the resonance-stabilized **3** *via* hydrolysis of the triazine ring. In addition, a gradual increase in the amount of **6** was observed with maximum levels at ~15% after 30 days. This degradate may have been the result of cyclization of acetylbiuret (stemming from the hydrolysis of **3**) or it may have arisen indirectly from **5**. The degradation kinetics, however, suggests that **6** originates from the direct hydrolysis of **2** resulting in elimination of the phenylsulfonylurea portion of the molecule.

In order to establish the identities of the components in the hydrolysis study, co-chromatography and mass spectral comparisons were made between the authentic reference standards and the hydrolysis degradates. At the time of the study, reference standards were available for all observed degradates with the exception of a relatively non-polar component which eluted from the HPLC column with a retention time of ~30 min (Fig. 3). In order to generate enough material for a thorough spectral evaluation, a large-scale incubation was run at pH 5. TLC purification afforded sufficient material for mass spectral evaluation, although the [^{1}H]NMR of this purified material in deuteroacetonitrile still revealed the presence of a significant amount of impurities. Surprisingly, the desired material crystallized out of the deuteroacetonitrile solution and the [^{1}H]NMR spectrum of the filtrate showed impurities only. After removal of the filtrate (and evaporation of the residual acetonitrile), the white crystalline solid was dissolved in deuteromethanol and the [^{1}H]NMR of this purified unknown revealed an intact trifluoropropylphenyl moiety as evidenced by the aromatic region which was virtually identical to the aromatic region of prosulfuron (Fig. 4). In addition, the methylene protons of the trifluoropropyl group were intact, indicating no transformation of this aliphatic substituent. Degradative

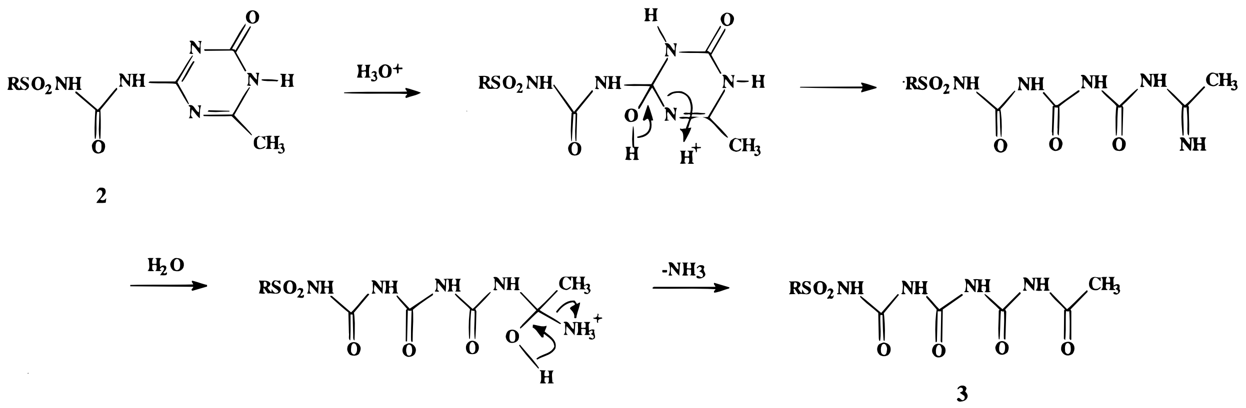

Fig. 6. Mechanism for the formation of **3**.

transformation did occur on the triazine portion of the molecule, as revealed by the loss of the methoxy protons and an upfield shift in the triazine methyl substituent in the phenylsulfonylacetamide derivative (δ 2·24) as compared to the parent molecule (δ 2·51). This upfield shift resulted from a loss of the triazine ring (and aromaticity) which originally provided a deshielded environment for the methyl protons.

The [^{1}H]NMR spectra of the recently synthesized **3** and the isolated, crystalline **3** differ slightly with respect to chemical shifts. This difference in chemical shifts is most evident for the aromatic protons and the acetamide methyl substituent. The aromatic protons in the isolated **3** are slightly upfield as compared to the synthesized reference standard. In addition, the acetamide methyl moiety is downfield (0·1 ppm) in the isolate as compared to the reference standard. It should be noted here that the crystallization of isolated **3** in the NMR solvent resulted in microgram quantities of the desired material, whereas milligram quantities of the synthesized standard was available for analysis. Additionally, any trace of acid and/or water could conceivably cause minor differences in the chemical shift.

Negative and positive ion Fast Atom Bombardment (FAB) MS analyses were performed on the isolated unknown degradate and each mode provided clear evidence that the component had a molecular weight of 424. Fragmentation was observed which corresponded to the loss of $(CONH)_2COCH_3$ ($m/z = 295$) and $(CONH)_3COCH_3$ ($m/z = 252$). In addition, electrospray LC-MS confirmed the molecular weight as 424. A pseudo MS/MS experiment was performed by increasing the orifice potential from -50 V to -100 V. At -100 V, characteristic fragmentation was observed at m/z 252 and 295. The LC-MS spectra for isolated **3** and the reference standard (synthesized and analyzed at a later date) are shown in Fig. 5. In order to obtain an exact molecular weight and, hence, an empirical formula, a high resolution, positive ion FAB spectrum was run. If one assumes that the unknown has an even number of nitrogens (even mass) and that the trifluoropropyl substituent is intact (as demonstrated by NMR), the empirical formula is $C_{14}H_{16}O_6N_4SF_3$. Although several structures could fit the empirical formula obtained above, the resonance-stabilized **3** proved to be the correct assignment based on LC/MS retention times, and mass spectra of isolated **3** and the authentic reference standard. The [^{1}H]NMR data support this conclusion, although slight chemical shift differences between isolated **3** and synthesized **3** exist.

In two contemporary aquatic metabolism studies (aerobic and anaerobic) with [*phenyl*-^{14}C] and [*triazine*-^{14}C]prosulfuron, the formation of resonance-stabilized **3** was also observed. Three additional degradates were postulated to arise from **3** by the sequential hydrolysis of acetic acid and each successive urea unit (Ciba, unpublished results). Confirmation of **3** in these aquatic studies, as well as the degradation/hydrolysis products of **3**, was made possible with the availability of the authentic reference standard for **3** and the standard for each degradation/hydrolysis product.

The postulated two-step mechanism for the hydrolytic opening of the triazine ring to form **3** is shown in Fig. 6. The first step involves hydrolysis of the triazine ring to form the resonance-stabilized amidine. The second step involves hydrolysis of the terminal amidine to give the acetamide **3**. This pathway was first suggested for propazine in 1964,[4] and only recently was structurally confirmed by comparison of the open-chain triazine product of thifensulfuron-methyl[5] with authentic standard prepared by hydrolysis of parent thifensulfuron-methyl in acidic solution. This work describes the identification of the triazine hydrolysis product **3** by comparison with the newly synthesized, authentic reference standard.

ACKNOWLEDGEMENTS

We thank Dr Luis Ruzo and Robert Toia of PTRL-West, Inc. for their valuable input into the structural elucidation of the open chain hydrolysis product of prosulfuron. Additional thanks to William Eberle of Ciba-Geigy Corporation for his expert NMR assistance.

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